Figure 1

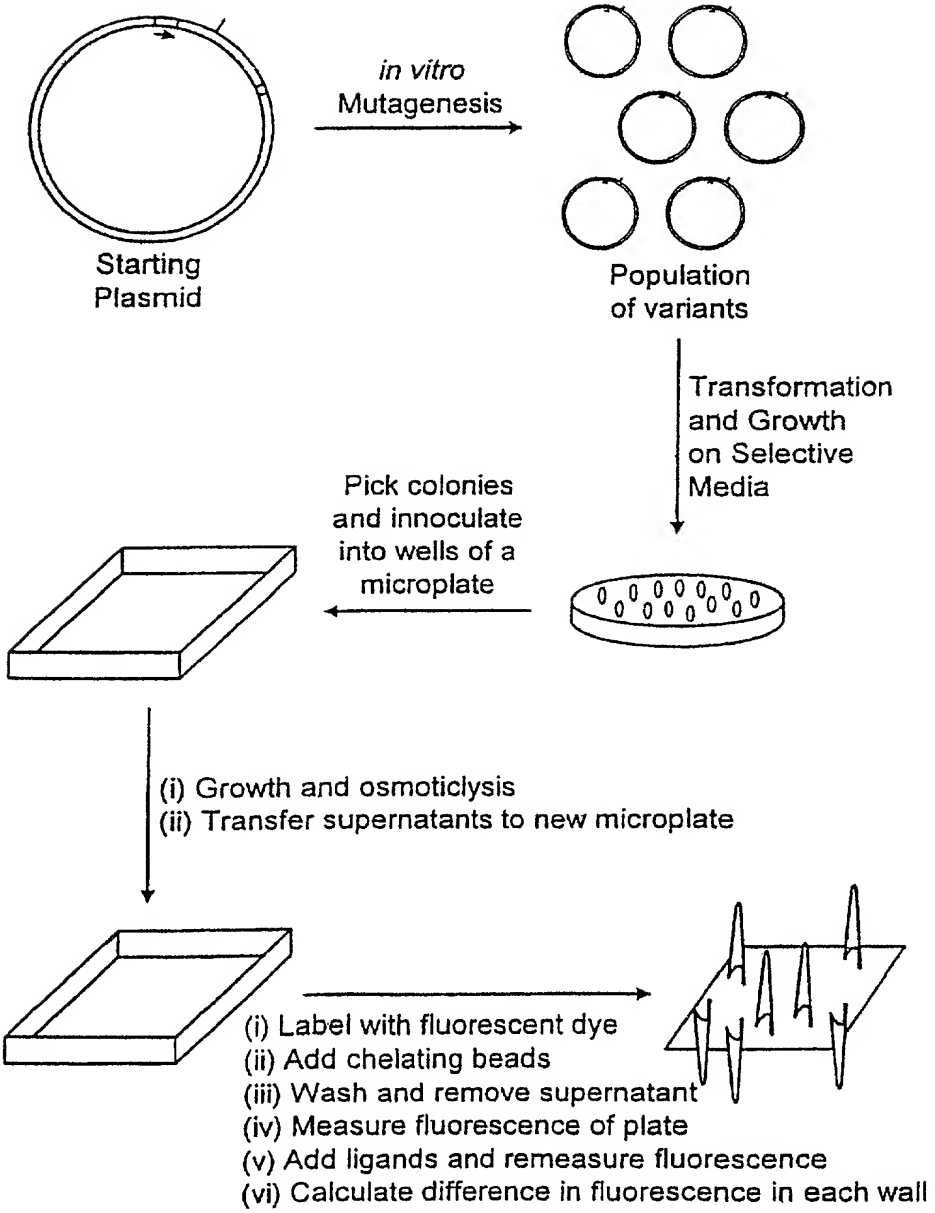

Starting Plasmid
in vitro Mutagenesis
Population of variants
Transformation and Growth on Selective Media
Pick colonies and innoculate into wells of a microplate
(i) Growth and osmoticlysis
(ii) Transfer supernatants to new microplate
(i) Label with fluorescent dye
(ii) Add chelating beads
(iii) Wash and remove supernatant
(iv) Measure fluorescence of plate
(v) Add ligands and remeasure fluorescence
(vi) Calculate difference in fluorescence in each wall

Figure 2

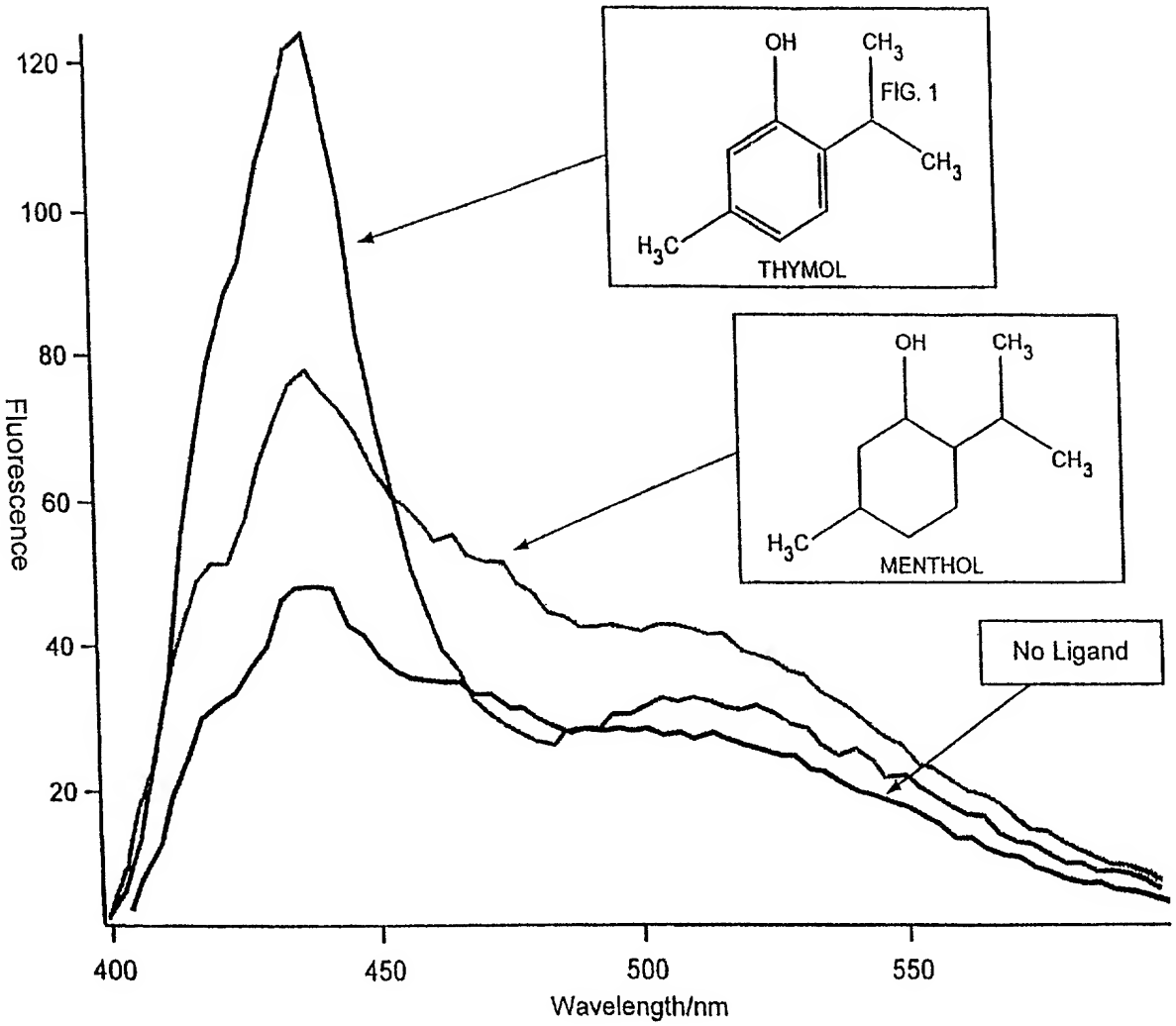

120
100
80
60
40
20
Fluorescence
400
450
500
550
Wavelength/nm
OH
CH3
FIG. 1
CH3
H3C
THYMOL
OH
CH3
CH3
H3C
MENTHOL
No Ligand

Figure 3

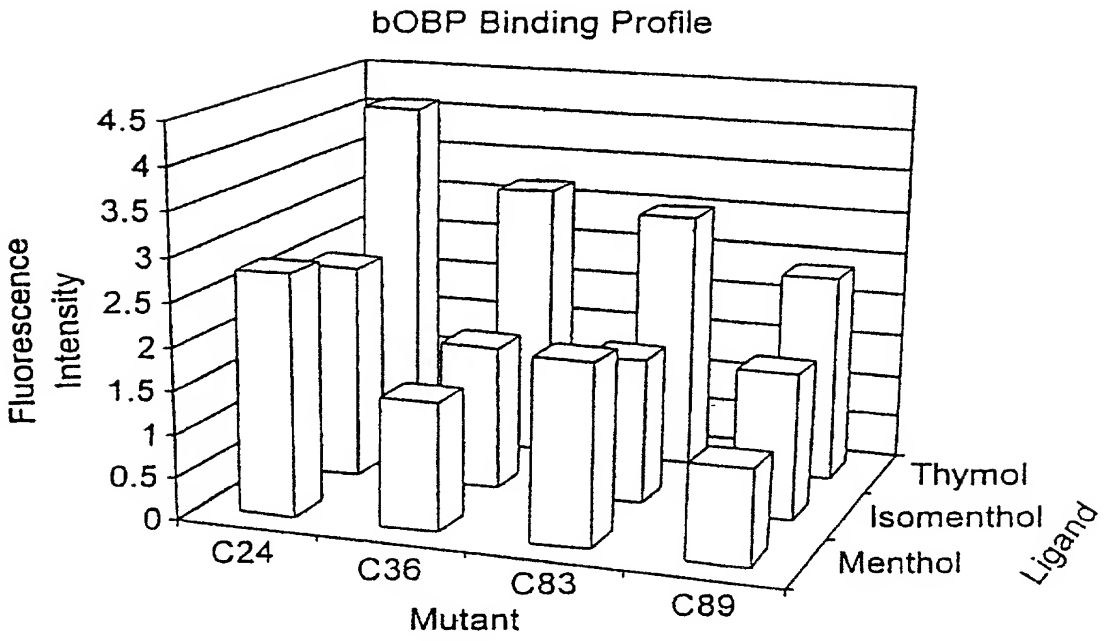

bOBP Binding Profile
Fluorescence Intensity
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
C24
C36
C83
C89
Mutant
Thymol
Isomenthol
Menthol
Ligand

Figure 4

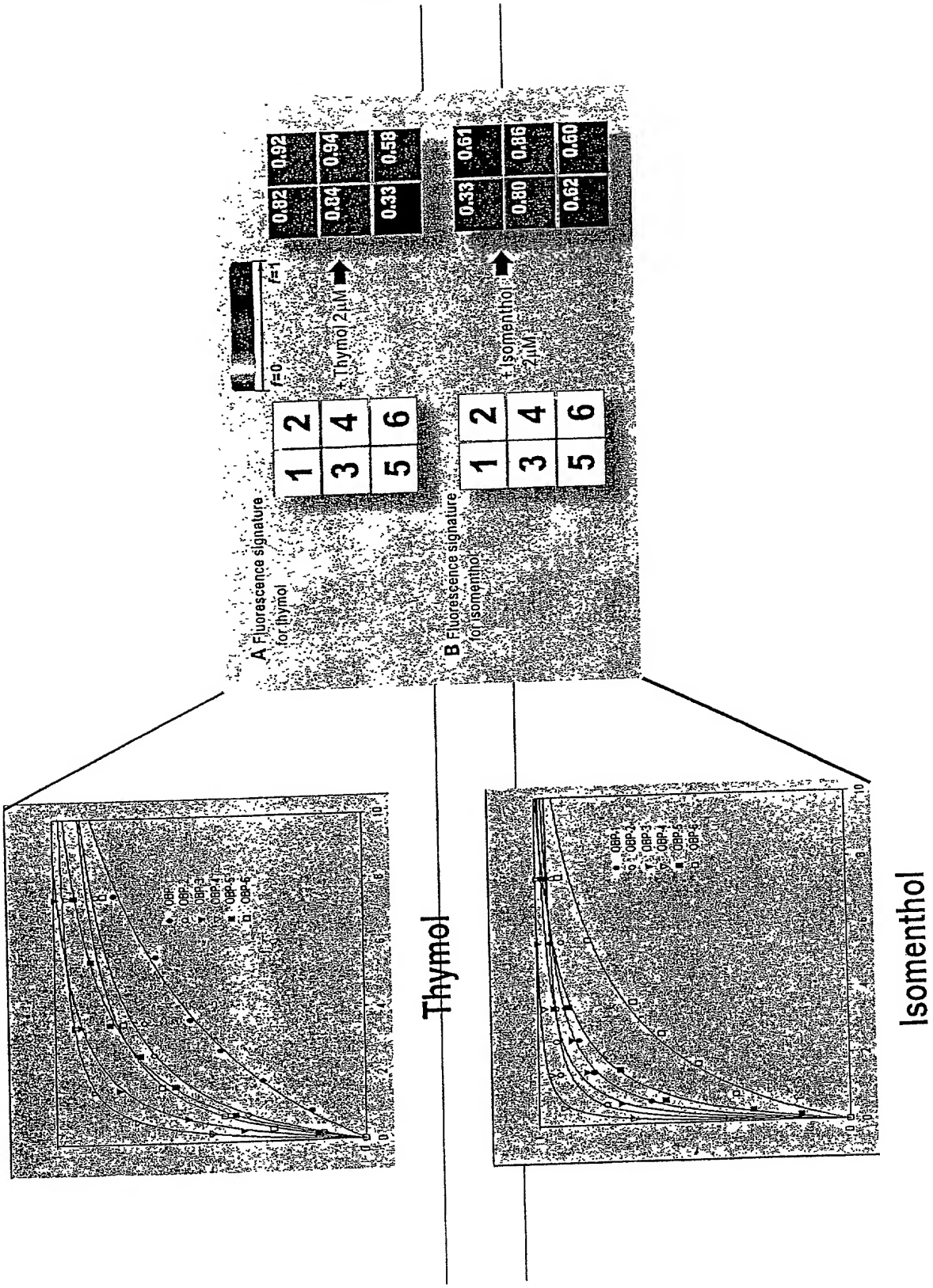

A Fluorescence signature for thymol
f=0
f=1
+ Thymol 2µM
0.92
0.94
0.58
0.82
0.84
0.33
1 2
3 4
5 6
B Fluorescence signature for isomenthol
+ Isomenthol 2µM
0.61
0.86
0.60
0.33
0.80
0.62
1 2
3 4
5 6
OBP1
OBP2
OBP3
OBP4
OBP5
OBP6
Thymol
Isomenthol

Figure 5A

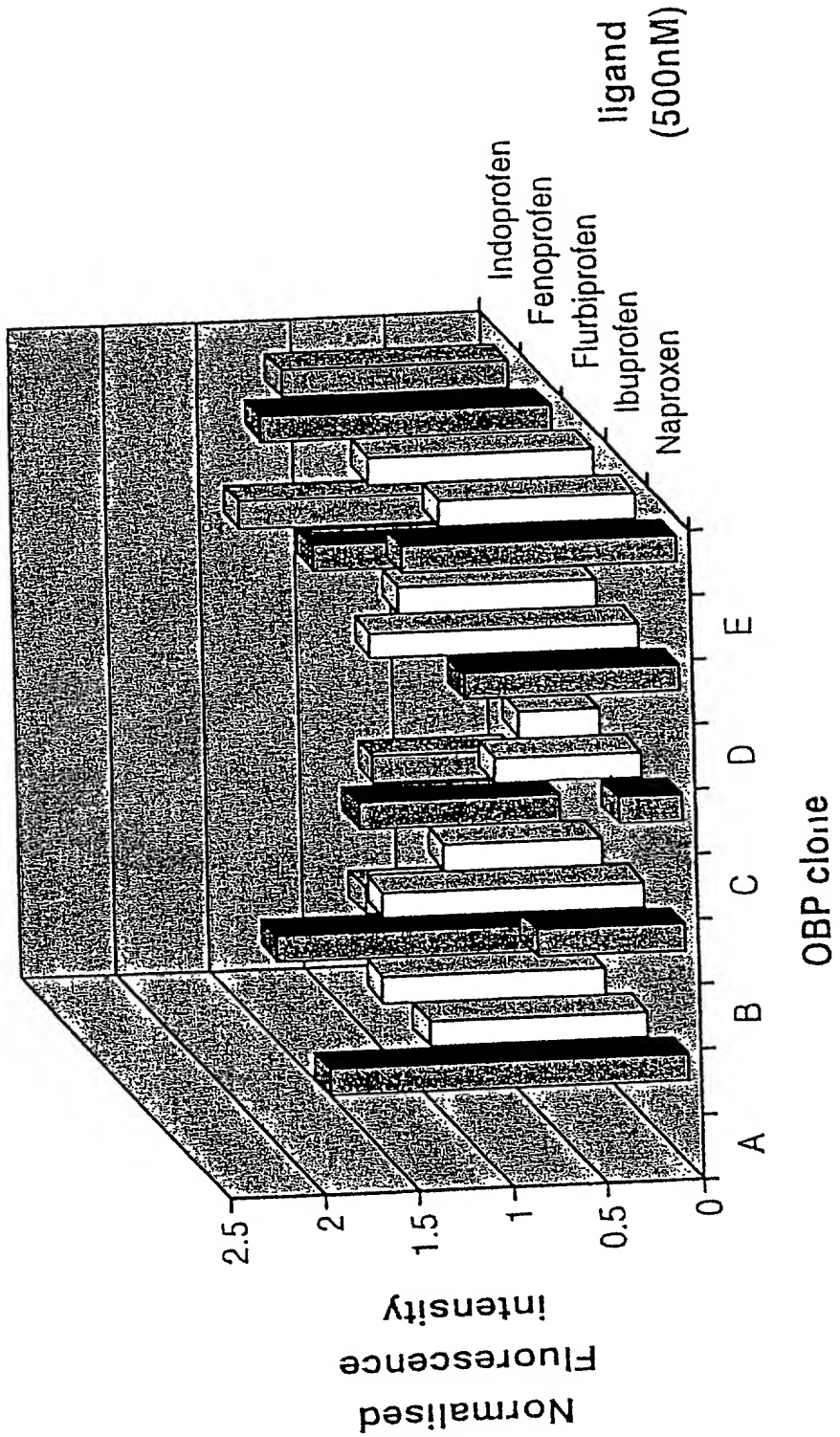
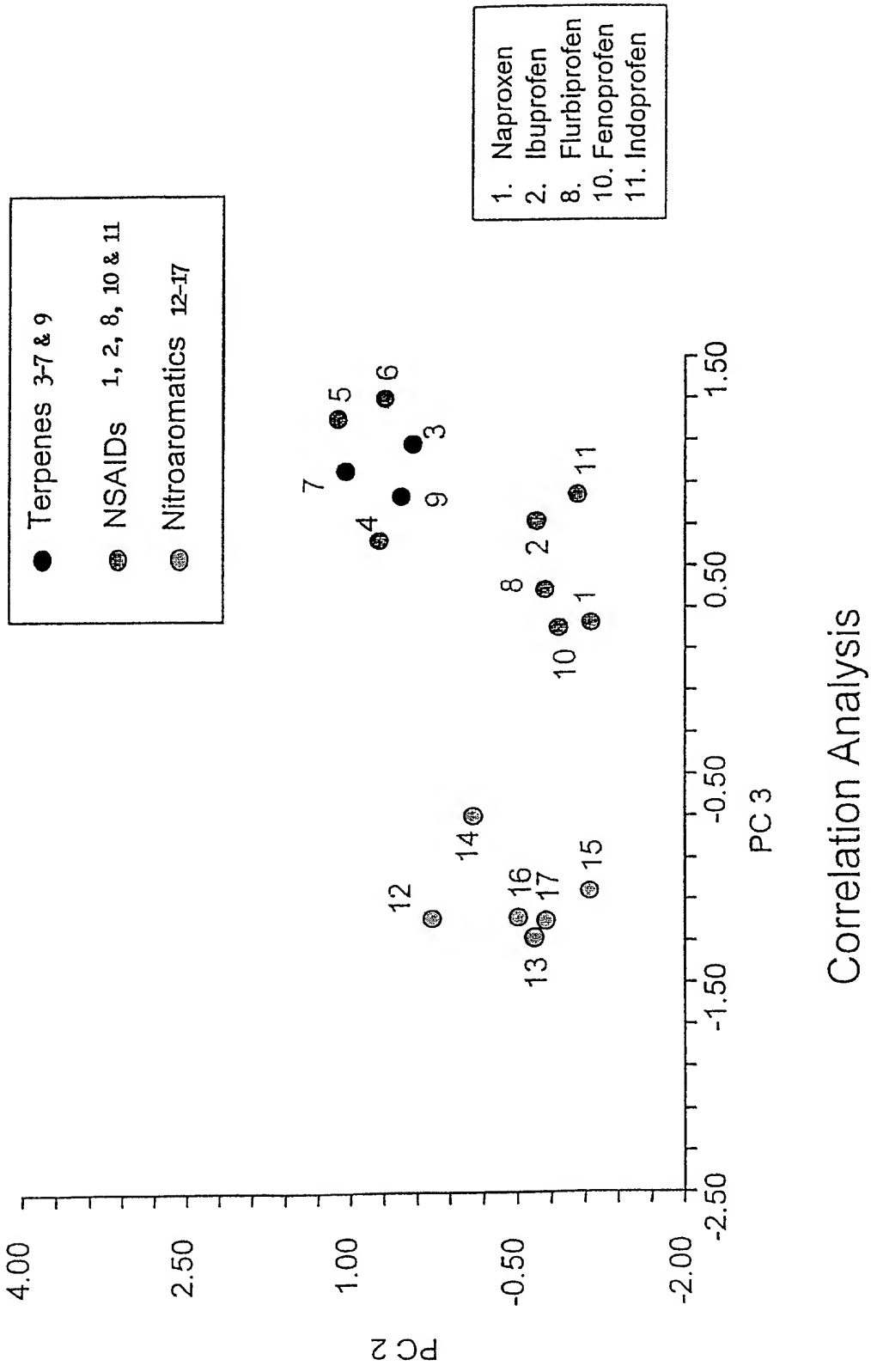

Normalised
Fluorescence
intensity
2.5
2
1.5
1
0.5
0
A
B
C
D
E
OBP clone
ligand
(500nM)
Indoprofen
Fenoprofen
Flurbiprofen
Ibuprofen
Naproxen

Figure 5B


Terpenes 3-7 & 9
NSAIDs 1, 2, 8, 10 & 11
Nitroaromatics 12-17
1. Naproxen
2. Ibuprofen
8. Flurbiprofen
10. Fenoprofen
11. Indoprofen
Correlation Analysis
PC 2
PC 3
4.00
2.50
1.00
-0.50
-2.00
-2.50
-1.50
-0.50
0.50
1.50